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ESSENTIAL OIL CHARACTERIZATION IN CLOVE (*Syzigium aromaticum* Linn.) BY GC-MS AND DETECTION OF ITS ADULTERATION BY ATR-FTIR METHOD

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ABSTRACT

Syzigium aromaticum **Linn. (clove) essential oil is known for its dental analgesic property, and traditionally used as antiseptic, carminative and as important condiment in foods. Clove essential oil has high medicinal values and its adulteration is done very often with cheap edible oils. The aim of the present study was to characterize the essential oil constituents and determine adulteration in essential oils isolated from the buds of clove. The essential oil composition was determined by GC-MS method and adulteration by ATR-FTIR method. GC-MS analysis of clove essential oil yielded 43 chemical compounds with eugenol (59.16%), *β*-selinene (9.34%), caryophyllene (7.68%), eugenol acetate (3.35%), and *α*-humulene (2.16%) as major compounds. The FTIR of isolated clove essential oil showed a characteristic peak at 1514 cm^{-1}, which is missing in market essential oil. The FTIR data of market clove essential oil matches with sesame oil. The clove essential oil was adulterated with sesame oil (0, 5, 10, 20, 30, 40, 50 and 100% *v/v*), and the FTIR resultant adulterated binary mixtures indicated distinct peaks at 2852.72 and 2924.09 cm^{-1} (for –C-H sp^3 & –C-H sp^2 stretching) that is absent in pure essential oil of clove. The present study provides a simple, economic, quick, reliable, and reproducible method for detection of adulteration in clove essential oil. GC-MS analysis further explored the chemical composition of clove essential oil collected from Erbil, Iraq.**

Keywords: Adulteration, ATR-FTIR, clove, GC-MS analysis, Myrtaceae, *Syzigium aromaticum*

INTRODUCTION

Syzigium aromaticum Linn. (Myrtaceae), commonly called clove, and its essential oil is well known dental analgesic easily available in pharmacies. *S. aromaticum* buds has traditionally been used as antiseptic, carminative and as important condiment in foods (Chaieb *et al.*, 2007; Haro-González *et al.*, 2021; El-Saber Batiha *et al.*, 2020). *S. aromaticum* essential oil and its bioactive compound eugenol reportedly is antibacterial (Ginting *et al.*, 2021; Radünz *et al.*, 2019), anti-inflammatory (Han and Parker, 2017), antioxidant (Teles et al., 2021), antifungal (Liu *et al.*, 2017), and antiviral agent (Brochot *et al.*, 2017). The *S. aromaticum* essential oil has high medicinal values and its adulteration with cheap edible oils is a very common practice. There are several hyphenated techniques available for detection and quantification of adulteration in essential oils such as GC-FID (gas chromatography-flame ionization detector), GC-MS (gas chromatography-mass spectroscopy), and ATR-FTIR [attenuated total reflectance-Fourier transform infrared] (Rodríguez *et al.*, 2018).

GC-MS is a highly sensitive, selective and accurate analytical tool for the identification and quantification of essential oil compounds. However, it has several drawbacks of being expensive, time consuming and requiring skilled person to handle the machine (Ahamad, 2021; Najibullah *et al.*, 2021). Hence, there is a need to search alternative analytical methods that could be easy, fast, reliable, sensitive, and accurate. The FTIR coupled with ATR (attenuated total reflectance) spectroscopy provides rapid, sensitive, and accurate alternative analytical method for the analysis of essential oils (Rodríguez *et al.*, 2018). ATR-FTIR spectroscopy requires no sample preparation steps and provides a global fingerprint of a sample so can be considered as an alternative way for essential oil quantification and detection of adulteration (Dhoot *et al.*, 2009; Rodríguez *et al.*, 2018). Further, the peak intensity is proportional to the concentration of each compound of essential oil. Essential oils exhibit a complex IR spectrum due to the presence of a large number of constituents in it, hence the identification of particular essential oil becomes difficult. Every essential oil shows some unique IR peaks. *S. aromaticum* essential oil mainly contains eugenol and eugenol acetate as major bioactive compounds, and these chemical compounds have a unique IR peak at 1514 cm^{-1} for aromatic C=C bonds (Dhoot *et al.*, 2009; Goñi *et al.*, 2016). The aim of present study was to characterize the chemical constituents of *S. aromaticum* essential oil by GC-MS and then determine adulteration by ATR-FTIR method.

MATERIALS AND METHODS

Plant materials and chemicals

The flowering buds of *Syzigium aromaticum* Linn. (500 g) was collected from local market of Erbil, Iraq in November, 2021. The authenticity of plant species was done by Dr. Raad A Kaskoos, Faculty of Pharmacy, Hawler Medical University, Erbil, Iraq. The plant sample was kept in Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil, Iraq for future reference (voucher number: PRL/2021/17). The reagents and solvent used in the study were of analytical grade.

Isolation of S. aromaticum essential oil

The flowering buds of *S. aromaticum* (250 g) were grinded in mixture grinder and then hydro-distilled for 6 h in Clevenger apparatus. The isolated essential oil was collected in graduated tube and filtered over anhydrous sodium sulphate. Then essential oil of clove was stored at 4ºC in a refrigerator for further use.

GC-MS analysis and identification of chemical compounds

The essential oil composition of clove buds was determined by GC-MS (Ahamad, 2021). The sample was run on Agilent Bench Top GC-MS (Agilent Technologies, Wilmington, DE, USA) equipment, fitted with a capillary column of DB-5 glass (30 m × 0.25 mm i.d.; film thickness of 0.25 μm). Helium was used at a flow rate of 1 mL min^{-1} as the carrier gas. The temperature of oven was set at 50°C for 2 min and then isothermally kept for 2 min at 300°C, while the injector port was maintained at 280°C, ion source temperature: 250°C, and interface temperature 300°C. The clove essential oil (0.1 μL, in hexane) was injected and the split ratio was kept at 1:10. Data capture took place at 70 eV using scanning times of 1.5 sec in the mass range of 50-1000 amu and run time was kept upto 37 min. The chromatography and mass spectra were handled with Chem station software (Agilent Technologies, Wilmington, DE, USA). The individual peaks/ constituents were identified by comparison of their Kovats Index (K.I.) with those of literature (Marya *et al.*, 2012; Amelia *et al.*, 2017; Rodríguez *et al.*, 2018). Further, the identification of chemical constituents was done by comparison of the fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NIST, NBS 54 K.L, WILEY8 libraries and published literature (Ali, 2001; Adams, 2007; Marya *et al.*, 2012; Amelia *et al.*, 2017; Rodríguez *et al.*, 2018; Uchôa Lopes *et al.*, 2020). Quantification was done by external standard method using calibration curves generated by running GC analysis of representative compounds.

FTIR analysis of S. aromaticum essential oil

The purity of isolated clove essential oil was analyzed by ATR-FTIR (attenuated total reflectance-Fourier transform infrared). ATR-FTIR (IRAffinity-1S, Shimadzu, Japan) was utilized to record the IR spectra of clove essential oil. The spectra acquisition was performed in the spectral range of 400 to 4000 cm^{-1}. The isolated clove essential oil was placed on ATR surface and FTIR spectra recorded using 45 scans and 4 cm^{-1} resolutions. The ATR surface was cleaned using hexane and background scan recorded before each sample scan. The FTIR spectrum of isolated clove essential oil was searched in library of FTR-FTIR (IRAffinity-1S, Shimadzu, Japan) and reported. The FTIR data of isolated clove essential oil was also compared with market clove essential oil. The FTIR spectrum of market clove essential oil was searched in library, and on the basis of searched results the market clove oil was found sesame oil with searched score of 917.

Detection of adulteration in S. aromaticum essential oil by FTIR

The detection of adulteration in clove essential oil was performed by ATR-FTIR method. The sesame oil was selected as solvent of adulteration because of market clove oil contains sesame oil. For detection of adulteration in clove essential oil, different admixtures of various concentrations of clove essential oil with sesame oil (0, 5, 10, 20, 30, 40, 50 and 100% *v/v*) were prepared and FTIR spectra of each concentration were recorded.

RESULTS AND DISCUSSION

GC-MS analysis of S. aromaticum essential oil

The hydro-distillation of clove bud resulted in isolation of colourless essential oil, and the mean yield was 17.28 ± 1.53% *v/w*. The GC-MS analysis of clove essential oil yielded 43 chemical compounds which constituted 98.66% of the total essential oil (Table 1; Fig. 1). Eugenol (59.16%), β-selinene (9.34%), caryophyllene (7.68%), eugenol acetate (3.35%), α-humulene (2.16%), methyl salicylate (1.85%), 1-cubenol, *epi*-(1.47%), menthyl chavicol (1.38%), 1-methylhexyl acetate (1.11%), and borneol (1.34%) were found as the major chemical compounds in essential oil of clove bud. The other important chemical compounds identified in essential oil were caryophyllene oxide (0.99%), carvone (0.63%), benzyl acetate (0.56%), α-bisabolol (0.56%), isoeugenol (0.51%), camphor (0.51%), and α-amorphene (0.38%). The minor chemical compounds present in essential oil of clove are also presented in Table 1. Amelia *et al.* (2017) studied the chemical composition of clove essential oil from Java and Manado and reported higher quantity of eugenol content in Manado (74.64%) as compared to Java (55.60%) in clove essential oil. They also observed eugenol acetate, caryophyllene and α-humulene as major chemical components in clove essential oil. Marya *et al.* (2012) studied chemical composition of clove essential oil by GC-MS and reported high contents of eugenol (58.29%) and eugenol acetate (19.10%). Similary, Uchôa Lopes *et al.* (2020) reported significantly higher content of eugenol (84.63%) in essential oil of clove buds collected from Brazil. Our findings showed similar phytochemical trends in essential oil of clove buds with some variation in chemical constituents, which may be attributed to the variation in geographical variation (Hamad *et al.*, 2013; Ahamad *et al.*, 2021). The present study successfully characterized the chemical constituents present in the essential oil of clove buds collected from Erbil by GC-MS method.

Table 1: GC-MS analysis of *S. aromaticum* essential oil

S. No.	Chemical constituents	RT* (min)	KI#	Composition (%)
1.	Furfural	6.121	838	0.04
2.	2-Heptanone	7.087	868	0.33
3.	Camphene	7.442	946	0.25
4.	5-Hepten-2-one, 6-methyl	8.295	958	0.06
5.	Myrcene	8.349	983	0.10
6.	*β*-Ocimene, (Z)	8.837	1028	0.26
7.	Limonene	9.183	1030	0.12
8.	Eucalyptol	9.272	1033	0.38
9.	1-Methylhexyl acetate	9.383	1035	1.11
10.	*m*-Cymene	9.484	1037	0.26
11.	*β*-Ocimene, (*E*)	9.938	1038	0.04
12.	*γ*-Terpinene	10.644	1059	0.06
13.	2-Nonanone	11.156	1092	0.04
14.	(*E*)-4,8-Dimethylnona-1,3,7-triene	12.055	1116	0.63
15.	Camphor	12.162	1123	0.51
16.	Benzyl acetate	12.346	1165	0.56
17.	Borneol	12.463	1166	1.34
18.	Methyl salicylate	13.006	1192	1.85
19.	Eugenol	17.113	1335	59.16
20.	Caryophyllene	18.337	1406	7.68
21.	*α*-Humulene	18.820	1448	2.16
22.	Isoeugenol	18.922	1451	0.51
23.	9-*epi*-(*E*)-Caryophyllene	19.097	1464	0.10
24.	*α*-Amorphene	19.210	1482	0.38
25.	*β*-Selinene	19.538	1489	9.34
26.	Eremophilene	19.935	1492	0.09
27.	(*E*,*E*)-*α*-Farnesene	20.148	1504	0.26
28.	Eugenol acetate	20.700	1521	3.35
29.	*epi*-Globulol	20.830	1540	0.20
30.	Caryophyllene oxide	21.025	1581	0.99
31.	Dihydroconiferyl alcohol	21.229	1597	0.29
32.	1-Cubenol, *epi*-	21.447	1617	1.47
33.	Junenol	21.552	1622	0.30
34.	*α*-Bisabolol	21.731	1668	0.56
35.	Menthyl chavicol	22.011	1669	1.38
36.	*α*-Terpinyl acetate	22.214	1695	0.50
37.	Mescaline	22.643	1700	0.07
38.	Benzyl benzoate	22.815	1725	0.12
39.	Carvone	23.022	1731	0.63
40.	*δ*-Cadinene	24.038	1755	0.05
41.	*α*-Calacorene	32.807	1921	0.59
42.	Menthyl eugenol	35.408	1985	0.47
43.	Tricosane	36.121	2300	0.07

*RT (min): retention time; #KI: Kovats index

***FTIR analysis* of *S. aromaticum* essential oil**

The essential oil isolated from buds of clove by FTIR analysis were presented in Fig. 2. As proven by GC-MS study, the eugenol (59.16%) and eugenol acetate (3.35%) were the major chemical constituents and these constituents showed unique peak at 1514 cm^{-1} for aromatic C=C bonds (the characteristic IR peaks of eugenol and eugenol acetate are presented in Fig. 3) (Dhoot *et al.,* 2009; Goñi *et al.,* 2016; Rodríguez *et al.,* 2018), which was observed in the present study (Fig. 2A). In

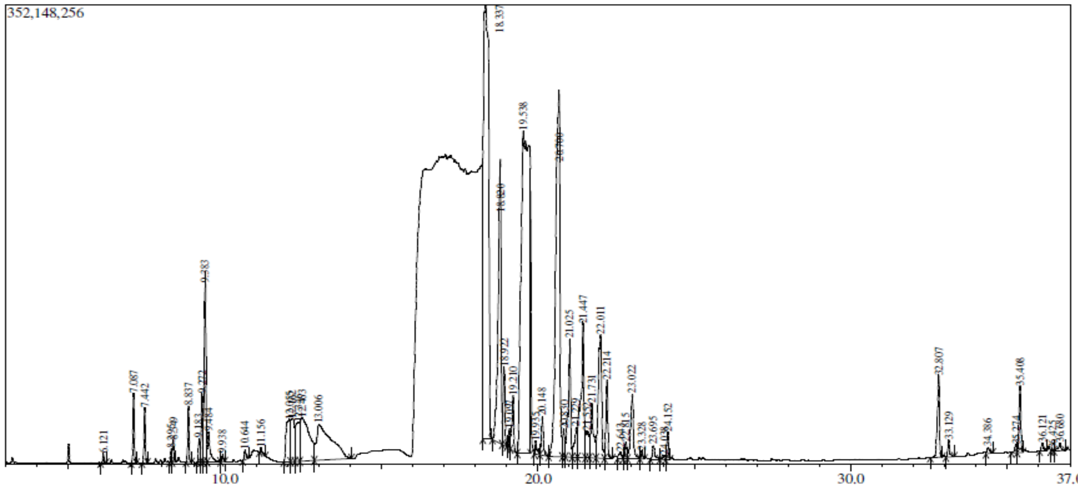

Fig. 1: GC/MS spectrum of *S. aromaticum* essential oil

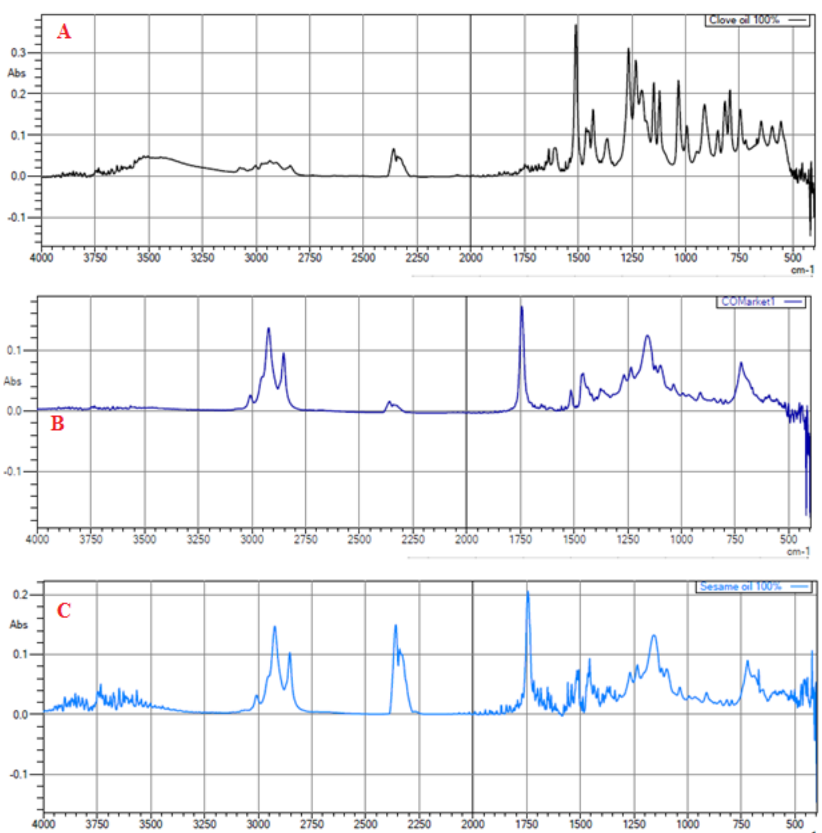

Fig. 2: FTIR absorption spectra of A) clove essential oil (100%); B) market clove essential oil; and C) sesame oil (100%)

Fig. 2, the results of FTIR analysis were presented: A) clove essential oil (100%); B) market clove essential oil; and C) sesame oil (100%). From Fig. 2 it is clear that peaks for eugenol and eugenol acetate at 1514 cm^{-1} were missing in market clove essential oil (Fig. 2B); and sesame oil (Fig. 2C). Also, in Fig. 2B & C at 2852.72 cm^{-1} strong peaks (for –C-H stretching, sp^3) with 669.236 and 779.614 area in market clove essential oil, and sesame oil, respectively, were noted that was missing in pure clove essential oil isolated in laboratory. For further confirmation, the FTIR spectrum of market clove essential oil was searched in FTIR library, and the search result matched with sesame oil (searched score: 917). As evident from Fig. 2B & C, the market clove essential oil was found adulterated with sesame oil (almost similar FTIR spectra). Hence sesame oil was selected for further study to detect adulteration in clove essential oil.

Detection of adulteration in S. aromaticum essential oil by FTIR

On the basis of above results, the sesame oil was selected as solvent for adulteration in clove essential oil. The clove essential oil was adulterated with sesame oil (@ 0, 5, 10, 20, 30, 40, 50 and 100% *v/v*), and results are presented in Fig. 4. As evident from FTIR spectra (Fig. 2 & 4), it is quite difficult to differentiate the peaks of pure clove essential oil with adulterated oils with sesame oil by

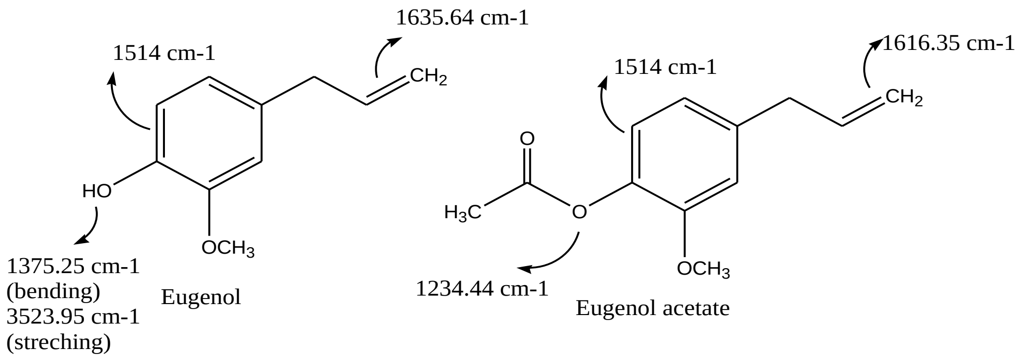

Fig. 3: Characteristic IR peaks of eugenol and eugenol acetate present in *S. aromaticum* essential oil

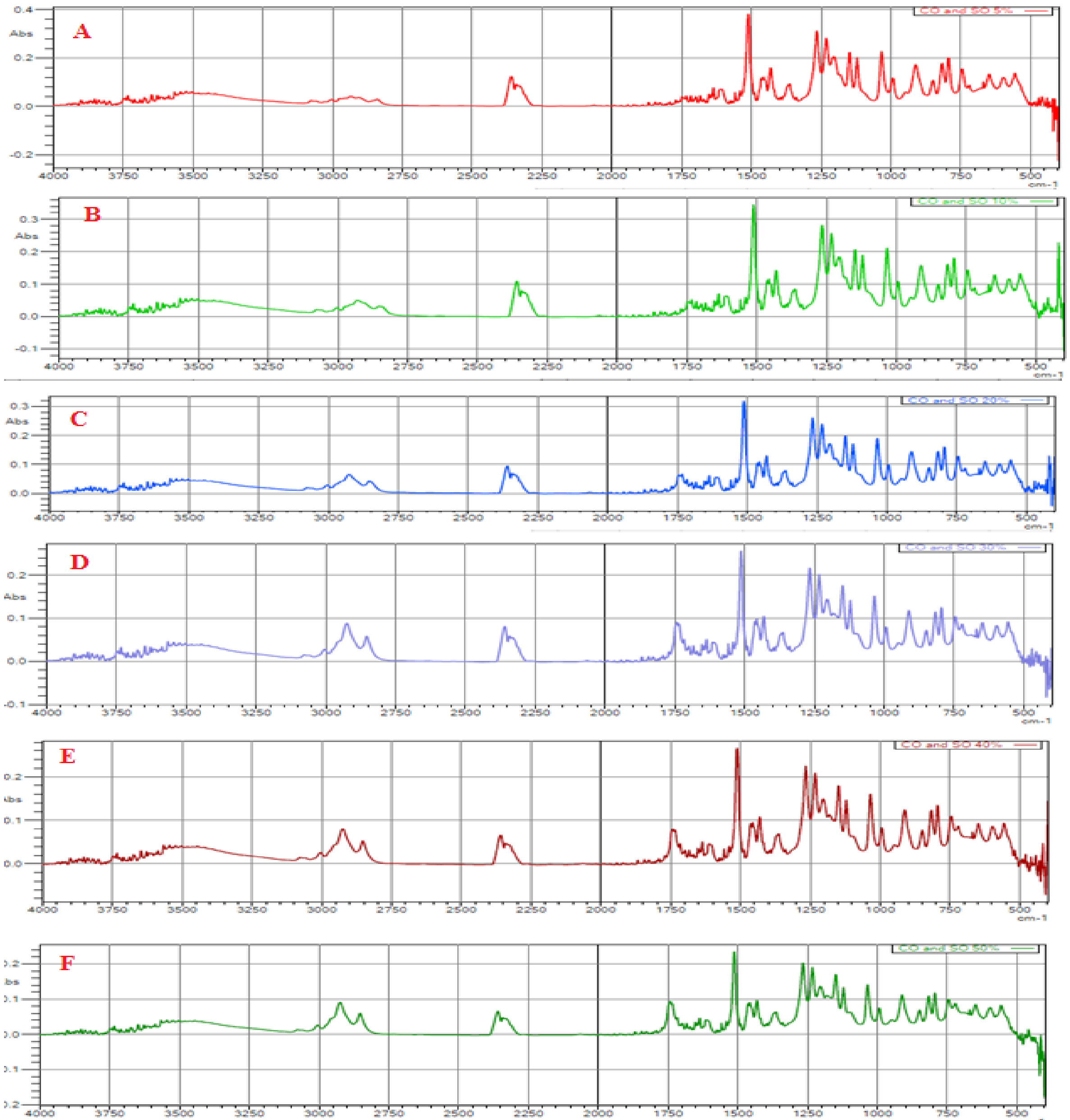

Fig. 4: FTIR absorption spectra of A) clove oil adulteration with sesame oil (5% *v/v*); B) 10% *v/v*; C) 20% *v/v*; D) 30% *v/v*; E) 40% *v/v*; and F) 50% *v/v*.

just visual examination of their whole spectrum. However, a careful examination of FTIR spectra, peaks at 2852.72 and 2924.09 cm^{-1} (for –C-H sp^3 & –C-H sp^2 stretching) provide clear difference between pure clove oils with adulterated oils, because these peaks were present only in adulterated oil and not in pure clove essential oil (Fig. 4A-F). Fig. 3B clearly indicated strong peaks at 2852.72 and 2924.09 cm^{-1} (10% *v/v* sesame oil adulteration), and this trend was continuously observed in 20-50% adulteration with sesame oil (Fig. 4C-F). The characteristic peak for eugenol and eugenol acetate at 1514 cm^{-1} were observed in all adulteration levels (Fig. 4A-F). The proposed method was simple, economic, quick, reliable, and reproducible for detection of adulteration in clove essential oil.

Conclusions: Clove buds are widely used as spice in cooking of food items, and medicinal it is used as dental analgesic, antiseptic, and carminative. Due to its high medicinal value, adulteration is very common in its essential oil. The present study characterized the chemical constituents and provided a reliable method for the determination of adulteration in it. The GC-MS analysis of clove essential oil revealed the presence of eugenol, *β*-selinene, caryophyllene, eugenol acetate, and *α*-humulene as major compounds. The FTIR method was successfully applied for the detection of adulterants in essential oil of clove buds. The FTIR results showed that at 10% *v/v* adulteration with sesame oil, a distinct peak appeared at 2852.72 and 2924.09 cm^{-1} that is missing in pure clove essential oil. The study showed that the ATR-FTIR method was simple, quick, economic, and sensitive for detection of adulteration in clove essential oil.

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